WEST Search History

09/831 816

DATE: Thursday, May 22, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB; THES=ASSIGNEE; PLUR=YES; OP=ADJ			
L3	L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine	0	L3
L2	PSF	1455	L2
L1	peptichemio	4	L1

END OF SEARCH HISTORY

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L15              STR
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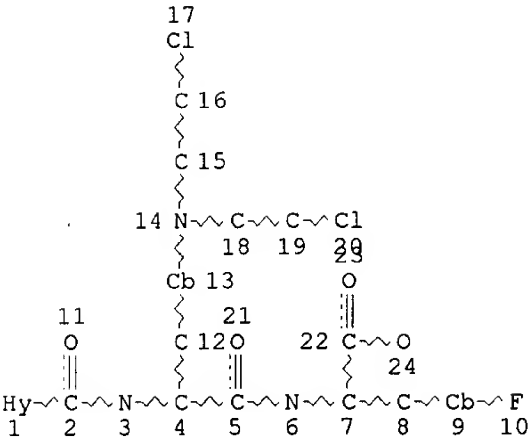

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GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS  24

STEREO ATTRIBUTES: NONE
L17           11 SEA FILE=REGISTRY SSS FUL L15
L18            6 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L17(L)PREP/RL


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L18  ANSWER 1 OF 6  HCAPLUS  COPYRIGHT 2003 ACS
ACCESSION NUMBER:         2001:923824  HCAPLUS
DOCUMENT NUMBER:          136:31672
TITLE:                    Melphalan derivatives, their preparation, and their
                          use as cancer chemotherapeutic drugs
INVENTOR(S):              Lewensohn, Rolf; Gullbo, Joakim; Larsson, Rolf;
                          Ehrsson, Hans; Luthman, Kristina
PATENT ASSIGNEE(S):       Oncopeptides AB, Swed.
SOURCE:                   PCT Int. Appl., 52 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
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WO 2001096367	A1	20011220	WO 2001-SE1318	20010611

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1290011	A1	20030312	EP 2001-938945	20010611

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

PRIORITY APPLN. INFO.:

SE 2000-2202	A	20000613
US 2000-211227P	P	20000613
WO 2001-SE1318	W	20010611

OTHER SOURCE(S): MARPAT 136:31672

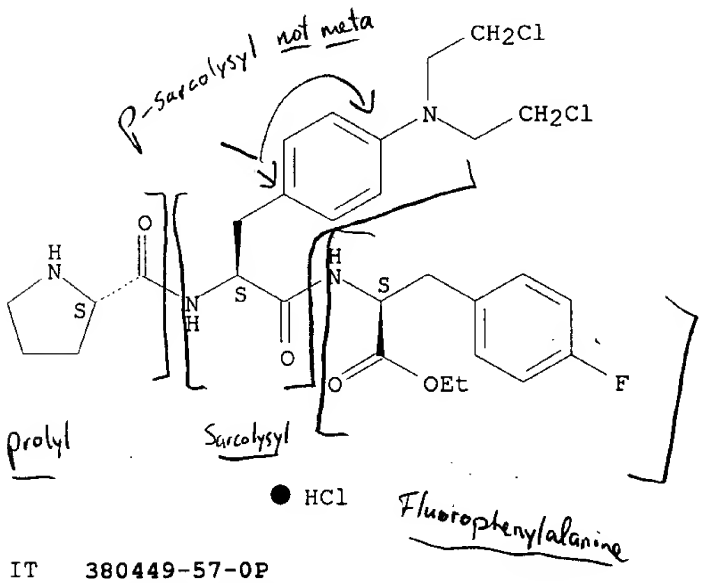
AB The invention provides alkylating di- and tripeptides based on a melphalan unit, and one or two addnl. amino acids or amino acid derivs., which can be used in the treatment of carcinogenic diseases. Further, the invention provides a pharmaceutical compn. comprising the alkylating peptides of the invention. Compd. prepn. is included.

IT **380449-56-9P**
RL: PAC (Pharmacological activity); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); **PREP (Preparation)**; USES (Uses)
(melphalan deriv. prepn. and use as cancer chemotherapeutic drugs)

RN 380449-56-9 HCAPLUS

CN L-Phenylalanine, L-prolyl-4-[bis(2-chloroethyl)amino]-L-phenylalanyl-4-fluoro-, ethyl ester, monohydrochloride (9CI) (CA INDEX NAME)

Absolute stereochemistry.



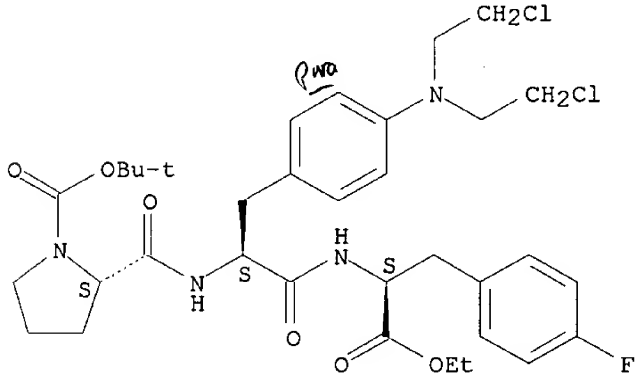
IT **380449-57-0P**
RL: RCT (Reactant); SPN (Synthetic preparation); **PREP (Preparation)**; RACT (Reactant or reagent)

(prepn. and reaction; melphalan deriv. prepn. and use as cancer chemotherapeutic drugs)

RN 380449-57-0 HCAPLUS

CN L-Phenylalanine, 1-[(1,1-dimethylethoxy)carbonyl]-L-prolyl-4-[bis(2-chloroethyl)amino]-L-phenylalanyl-4-fluoro-, ethyl ester (9CI) (CA INDEX NAME)

Absolute stereochemistry.



REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 2 OF 6 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2000:368410 HCAPLUS

DOCUMENT NUMBER: 132:347949

TITLE: Method for producing L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine and derivatives thereof

INVENTOR(S): Mehlem, Francesco; Di Vittorio, Pietro

PATENT ASSIGNEE(S): Peptichemio A.-G., Switz.

SOURCE: PCT Int. Appl., 20 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: German

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000031119	A1	20000602	WO 1998-CH498	19981119
W: AU, CA, HU, IL, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9910193	A1	20000613	AU 1999-10193	19981119
EP 1129107	A1	20010905	EP 1998-952496	19981119
R: AT, BE, ES, NL, SE				
JP 2002530428	T2	20020917	JP 2000-583946	19981119

PRIORITY APPLN. INFO.: WO 1998-CH498 A 19981119

OTHER SOURCE(S): CASREACT 132:347949; MARPAT 132:347949

AB An improved synthesis of the title compd., a component of the chemotherapeutic mixt. Peptichemio, and its alkyl esters or acid addn. salts, is claimed. Thus, C-terminal protected L-p-fluorophenylalanine was

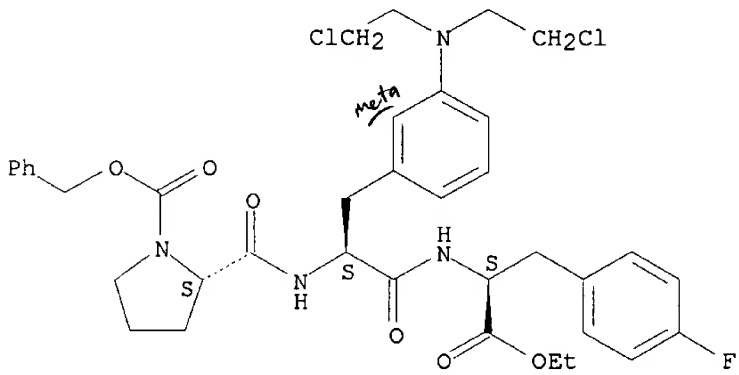
reacted with N-protected L-m-sarcolysine in the presence of dicyclohexylcarbodiimide, to give N,C-protected L-m-sarcolysyl-L-p-fluorophenylalanine. The N-protecting group was removed, to give C-protected L-m-sarcolysyl-L-p-fluorophenylalanine, which was then reacted with N-protected proline, to give N,C-protected L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine. Finally the N-protecting group was removed and the HCl salt was prepd. to give Et L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanate hydrochloride in 5% yield.

IT **39064-36-3P**
RL: RCT (Reactant); SPN (Synthetic preparation); **PREP (Preparation)**; RACT (Reactant or reagent)
(prepn. of L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine for use as chemotherapeutic agents)

RN 39064-36-3 HCAPLUS

CN L-Phenylalanine, 1-[(phenylmethoxy)carbonyl]-L-prolyl-3-[bis(2-chloroethyl)amino]-L-phenylalanyl-4-fluoro-, ethyl ester (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (-).

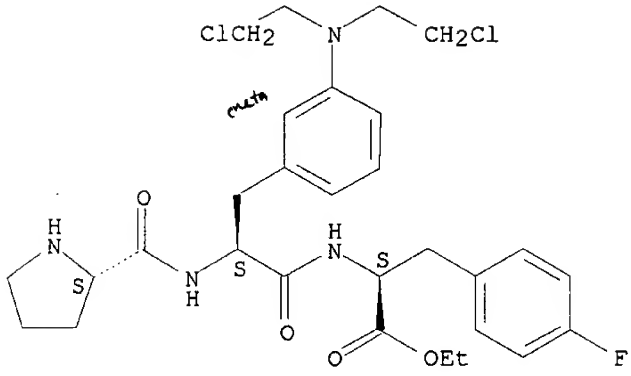


IT **35849-47-9P**
RL: SPN (Synthetic preparation); **PREP (Preparation)**
(prepn. of L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine for use as chemotherapeutic agents)

RN 35849-47-9 HCAPLUS

CN L-Phenylalanine, L-prolyl-3-[bis(2-chloroethyl)amino]-L-phenylalanyl-4-fluoro-, ethyl ester, monohydrochloride (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (-).



● HCl

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 3 OF 6 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:64700 HCAPLUS
DOCUMENT NUMBER: 130:144179
TITLE: Pharmaceutical composition containing Peptichemio for cancer treatment
INVENTOR(S): Mehlem, Francesco
PATENT ASSIGNEE(S): Peptichemio A.-G., Switz.
SOURCE: PCT Int. Appl., 20 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9902177	A1	19990121	WO 1998-CH300	19980707
W: AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, GH, GM, GW, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9879049	A1	19990208	AU 1998-79049	19980707
EP 1001799	A1	20000524	EP 1998-929194	19980707
EP 1001799	B1	20011031		
R: CH, DE, FR, GB, IT, LI				
JP 2001509487	T2	20010724	JP 2000-501767	19980707
EP 1132395	A2	20010912	EP 2001-201272	19980707
EP 1132395	A3	20020206		
R: CH, DE, FR, GB, IT, LI				

PRIORITY APPLN. INFO.: CH 1997-1651 A 19970707
EP 1998-929194 A3 19980707
WO 1998-CH300 W 19980707

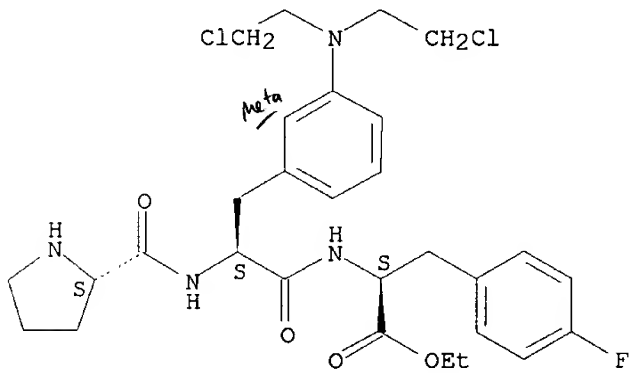
AB Peptichemio, a mixt. of 6 synthetic peptides each contg. L-m-sarcolysin, shows anticancer activity, esp. against melanomas. The peptides, and their lower alkyl esters and /or acid addn. salts, are formulated as delayed-release compns. with a cyclodextrin as carrier to provide adequate bioavailability over an extended period. Thus, synthesis of 1 of the peptides, L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine Et ester hydrochloride (I), from N-carbobenzoxy-L-proline, N-carbobenzoxy-L-m-sarcolysin, and L-p-fluorophenylalanine Et ester by the DCCD method is described. Oral cytostatic capsules contained I 12 mg and .beta.-cyclodextrin 25 g.

IT **35849-47-9P**
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); **PREP (Preparation)**; USES (Uses)
(pharmaceutical compn. contg. Peptichemio for cancer treatment)

RN 35849-47-9 HCAPLUS

CN L-Phenylalanine, L-prolyl-3-[bis(2-chloroethyl)amino]-L-phenylalanyl-4-fluoro-, ethyl ester, monohydrochloride (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (-).



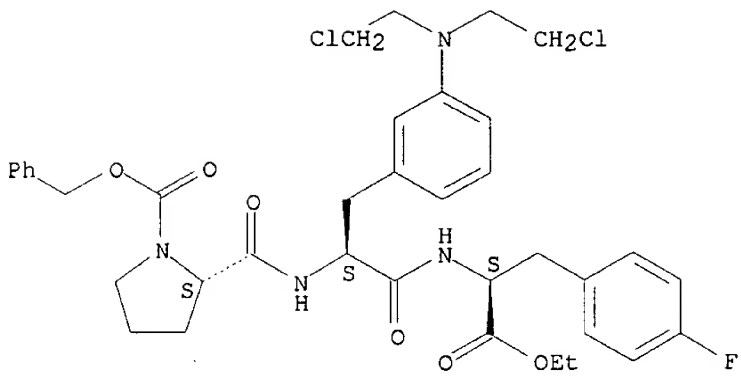
● HCl

IT **39064-36-3P**
RL: RCT (Reactant); SPN (Synthetic preparation); **PREP (Preparation)**; RACT (Reactant or reagent)
(pharmaceutical compn. contg. Peptichemio for cancer treatment)

RN 39064-36-3 HCAPLUS

CN L-Phenylalanine, 1-[(phenylmethoxy)carbonyl]-L-prolyl-3-[bis(2-chloroethyl)amino]-L-phenylalanyl-4-fluoro-, ethyl ester (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (-).



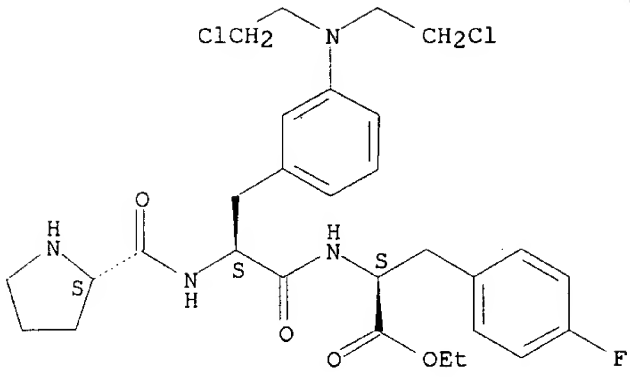
REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 4 OF 6 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1973:72600 HCAPLUS
DOCUMENT NUMBER: 78:72600
TITLE: Tetracycline derivatives of synthetic m-[bis(2-chloroethyl)amino]-L-phenylalanine-containing oligopeptides
INVENTOR(S): De Barbieri, Augusto
PATENT ASSIGNEE(S): Istituto Sieroterapico Serafino Belfanti
SOURCE: Ger. Offen., 37 pp.
CODEN: GWXXBX
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 2128623	A1	19730104	DE 1971-2128623	19710609

PRIORITY APPLN. INFO.: DE 1971-2128623 19710609
GI For diagram(s), see printed CA Issue.
AB [In this abstr. FPhe = p-fluoro-L-phenylalanyl, ClPhe = m-[bis(2-chloroethyl)amino]-L-phenylalanyl, EtAsp = .beta.-ethyl-L-aspartyl.] The title compds. (I; R = FPhe-ClPhe-Asn-OEt, Pro-ClPhe-FPhe-OEt, Pro-ClPhe-Nva-OEt, Ser-FPhe-ClPhe-OEt, FPhe-EtAsp-ClPhe-OEt, FPhe-Gly-ClPhe-Nva-OEt) were prepd. by treating tetracycline with H2CO and the corresponding peptide. I caused Sarcoma 180 tumor regression.
IT **35849-47-9P 39064-35-2P 39064-36-3P**
RL: SPN (Synthetic preparation); **PREP (Preparation)**
(prepn. of)
RN 35849-47-9 HCAPLUS
CN L-Phenylalanine, L-prolyl-3-[bis(2-chloroethyl)amino]-L-phenylalanyl-4-fluoro-, ethyl ester, monohydrochloride (9CI) (CA INDEX NAME)

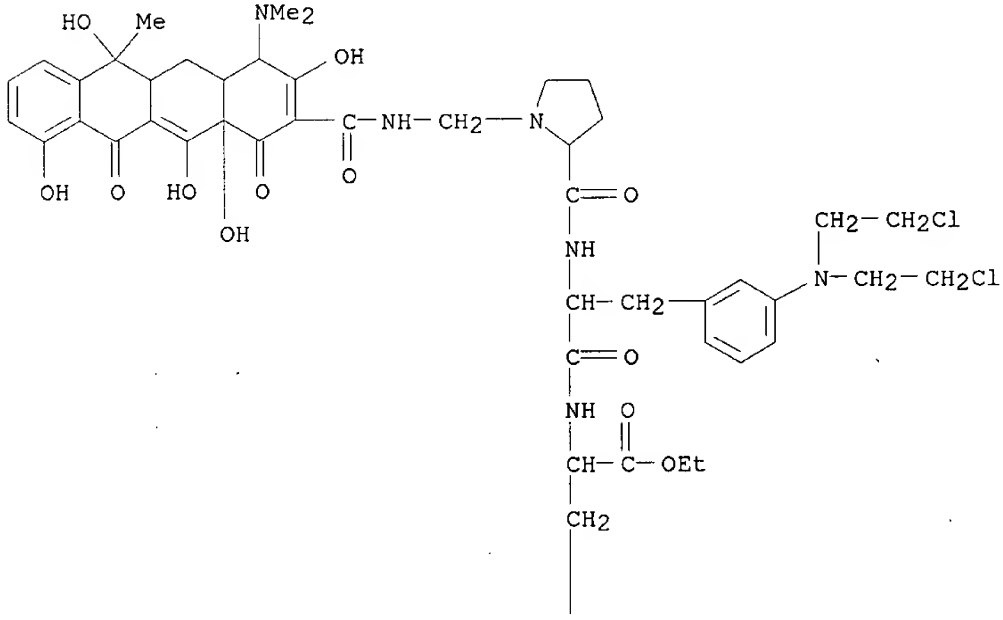
Absolute stereochemistry. Rotation (-).



● HCl

RN 39064-35-2 HCAPLUS
CN L-Phenylalanine, N-[3-[bis(2-chloroethyl)amino]-N-[1-[[[[4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenyl]carbonyl]amino]methyl]-L-prolyl]-L-phenylalanyl]-4-fluoro-, ethyl ester, monohydrochloride, [4S-(4.alpha.,4a.alpha.,5a.alpha.,6.beta.,12a.alpha.)]- (9CI) (CA INDEX NAME)

PAGE 1-A



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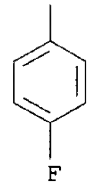
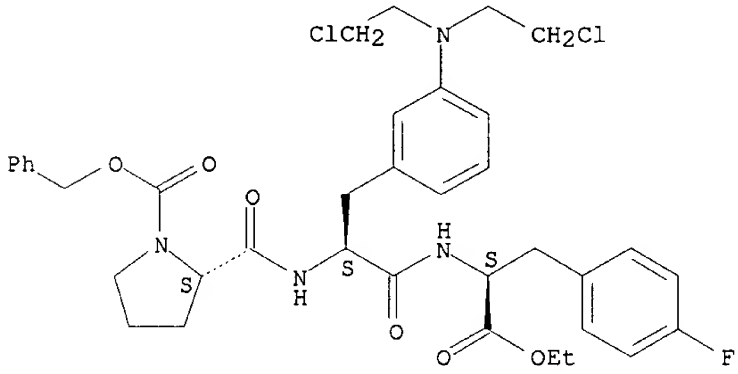


● HCl

RN 39064-36-3 HCAPLUS
CN L-Phenylalanine, 1-[(phenylmethoxy)carbonyl]-L-prolyl-3-[bis(2-chloroethyl)amino]-L-phenylalanyl-4-fluoro-, ethyl ester (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (-).



L18 ANSWER 5 OF 6 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1973:30201 HCAPLUS
DOCUMENT NUMBER: 78:30201
TITLE: Tetracycline-containing peptides with antitumor activity
PATENT ASSIGNEE(S): Istituto Sieroterapico Milanese "Serafino Belfanti" Ente Morale
SOURCE: Fr. Demande, 28 pp.
CODEN: FRXXBL
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
FR 2101226		19720505		
PRIORITY APPLN. INFO.:			IT 1970-28334	19700805

GI For diagram(s), see printed CA Issue.

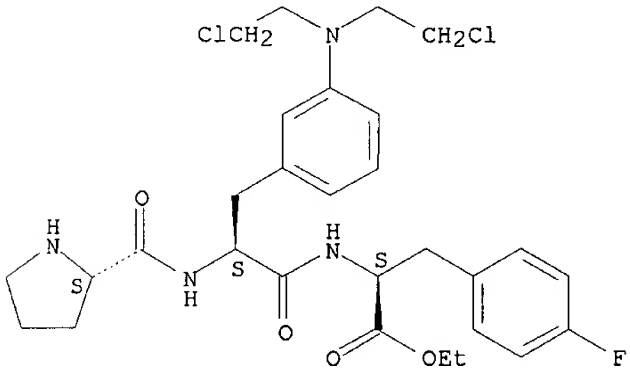
AB Tetracycline derivs. (I) in which R is a di-, tri, or tetrapeptide contg. the m-[bis(2-chloroethyl)amino]phenylalanine residue were prepd. by the Mannich reaction of tetracycline with the appropriate peptide. The necessary peptides were prepd. by the dicyclohexylcarbodiimide procedure.

IT **35849-47-9P 39064-35-2P 39064-36-3P**
RL: SPN (Synthetic preparation); **PREP (Preparation)**
(prepn. of)

RN 35849-47-9 HCAPLUS

CN L-Phenylalanine, L-prolyl-3-[bis(2-chloroethyl)amino]-L-phenylalanyl-4-fluoro-, ethyl ester, monohydrochloride (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (-).

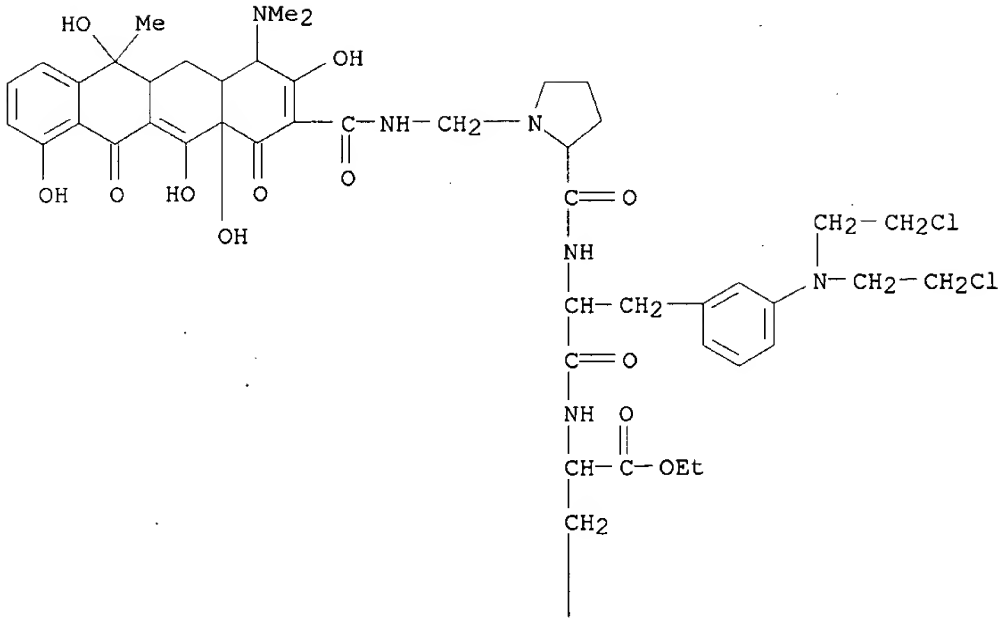


● HCl

RN 39064-35-2 HCAPLUS

CN L-Phenylalanine, N-[3-[bis(2-chloroethyl)amino]-N-[1-[[[[4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenyl]carbonyl]amino]methyl]-L-prolyl]-L-phenylalanyl]-4-fluoro-, ethyl ester, monohydrochloride, [4S-(4.alpha.,4a.alpha.,5a.alpha.,6.beta.,12a.alpha.)]- (9CI) (CA INDEX NAME)

PAGE 1-A



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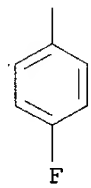
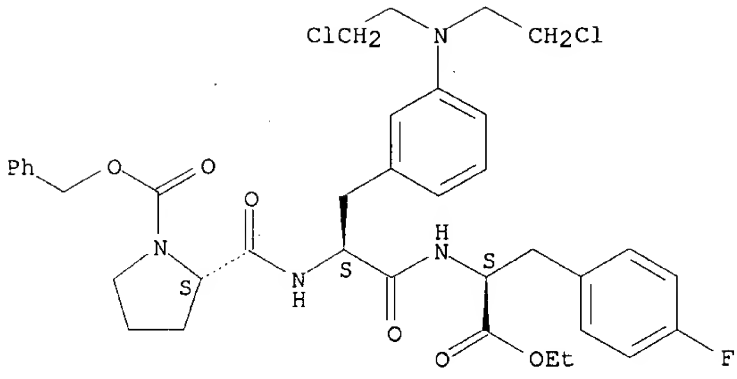


● HCl

RN 39064-36-3 HCAPLUS
CN L-Phenylalanine, 1-[(phenylmethoxy)carbonyl]-L-prolyl-3-[bis(2-chloroethyl)amino]-L-phenylalanyl-4-fluoro-, ethyl ester (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (-).



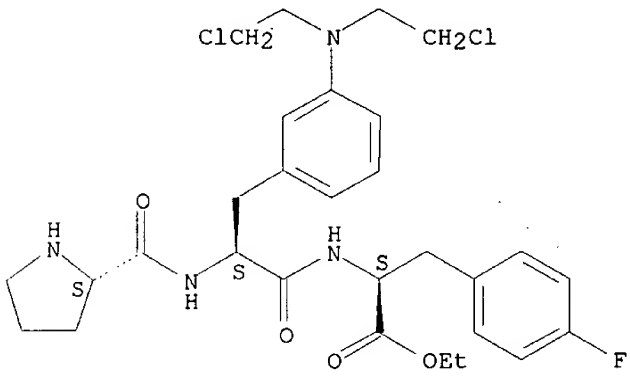
L18 ANSWER 6 OF 6 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1972:86148 HCAPLUS
DOCUMENT NUMBER: 76:86148
TITLE: Cytostatic m-[bis(2-chloroethyl)amino]-L-phenylalanine-containing oligopeptides
INVENTOR(S): De Barbieri, Agusto
PATENT ASSIGNEE(S): Istituto Sieroterapico Serafino Belfanti
SOURCE: Ger. Offen., 53 pp.
CODEN: GWXXBX
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 2128549	A	19720113	DE 1971-2128549	19710609
DE 2128549	B2	19760129		
DE 2128549	C3	19760909		
FR 2094175	A1	19720204	FR 1970-29723	19700812
FR 2094175	A5	19720204		

PRIORITY APPLN. INFO.: US 1970-45585 19700611
GI For diagram(s), see printed CA Issue.
AB (Y = -HNCH[CH2C6H4N(CH2CH2Cl)2]CO-; -QPhe- = -HNCH-(CH2C6H4F-p)CO-; NArg = N.omega.-nitro-L-arginyl; Z = PhCH2O2C). The title compds. [I; R = H, H-QPhe; Pro; Ser-QPhe, H-QPhe-(EtO)Asp, or HCO-QPhe; R1 = Asp-OEt, OEt, QPhe-OEt, Nva-OEt, Lys-OEt, Lys-Nva-OEt, Lys-QPhe-OEt, N-Arg-Nva-OEt, NArg-QPhe-OEt, or Arg-Lys-QPhe-His-OH] were prepd. and used as cytostatics according to Cancer Chemotherapy National Service Center methods esp. against Sarcoma 180 and Adenocarcinoma 755 in mice and addnl. clinically against several tumors. Thus, Z-Asp-OEt was hydrogenated over Pd/C in MeOH-AcOH, HCl added, the base released in DMF, and ZyOH and dicylohexylcarbodiimide added to 0.degree. to give 79% ZYAsp-OEt. The Z group was removed by hydrogenolysis in MeOH-HCl in the presence of Pd/C to give 75% I.HCl (R = H, R1 = Asp-OEt). Similarly prepd. and used were 16 other I.
IT **35849-47-9P**
RL: SPN (Synthetic preparation); **PREP (Preparation)**
(prepn. of)
RN 35849-47-9 HCAPLUS

CN L-Phenylalanine, L-prolyl-3-[bis(2-chloroethyl)amino]-L-phenylalanyl-4-fluoro-, ethyl ester, monohydrochloride (9CI) (CA INDEX NAME)

Absolute stereochemistry. Rotation (-).



● HCl

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=> d que
L21             STR
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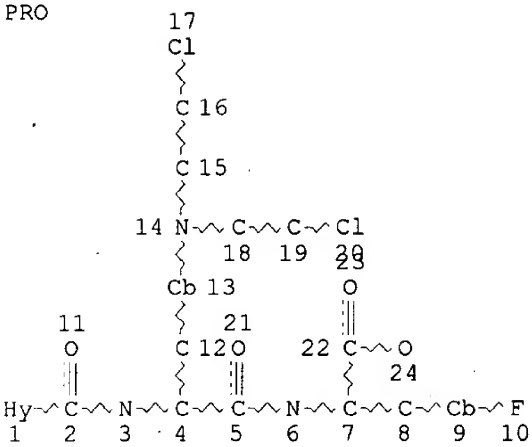
PRO

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L23  ANSWER 1 OF 1  CASREACT  COPYRIGHT 2003 ACS
ACCESSION NUMBER:         132:347949  CASREACT
TITLE:                    Method for producing L-prolyl-L-m-sarcolysyl-L-p-
                          fluorophenylalanine and derivatives thereof
INVENTOR(S):              Mehlem, Francesco; Di Vittorio, Pietro
PATENT ASSIGNEE(S):       Peptichemio A.-G., Switz.
SOURCE:                   PCT Int. Appl., 20 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 German
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.        KIND  DATE          APPLICATION NO.  DATE
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     WO 2000031119      A1   20000602      WO 1998-CH498    19981119
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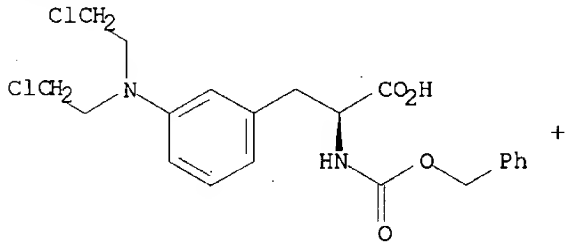
W: AU, CA, HU, IL, JP, US
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

AU 9910193	A1	20000613	AU 1999-10193	19981119
EP 1129107	A1	20010905	EP 1998-952496	19981119
R: AT, BE, ES, NL, SE				
JP 2002530428	T2	20020917	JP 2000-583946	19981119
PRIORITY APPLN. INFO.:			WO 1998-CH498	19981119

OTHER SOURCE(S): MARPAT 132:347949

AB An improved synthesis of the title compd., a component of the chemotherapeutic mixt. Peptichemio, and its alkyl esters or acid addn. salts, is claimed. Thus, C-terminal protected L-p-fluorophenylalanine was reacted with N-protected L-m-sarcolysine in the presence of dicyclohexylcarbodiimide, to give N,C-protected L-m-sarcolysyl-L-p-fluorophenylalanine. The N-protecting group was removed, to give C-protected L-m-sarcolysyl-L-p-fluorophenylalanine, which was then reacted with N-protected proline, to give N,C-protected L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine. Finally the N-protecting group was removed and the HCl salt was prepd. to give Et L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanate hydrochloride in 5% yield.

RX(1) OF 1



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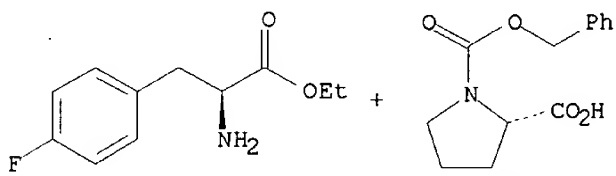

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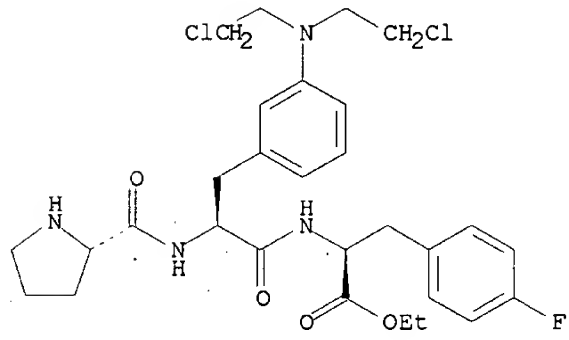
RX(1) OF 1



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REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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NEWS 5	Aug 19	Aquatic Toxicity Information Retrieval (AQUIRE) now available on STN
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NEWS 7	Sep 03	JAPIO has been reloaded and enhanced
NEWS 8	Sep 16	Experimental properties added to the REGISTRY file
NEWS 9	Sep 16	CA Section Thesaurus available in CAPLUS and CA
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NEWS 29	Mar 24	Additional information for trade-named substances without structures available in REGISTRY
NEWS 30	Apr 11	Display formats in DGENE enhanced
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NEWS 32	Apr 17	Polymer searching in REGISTRY enhanced
NEWS 33	Apr 21	Indexing from 1947 to 1956 being added to records in CA/CAPLUS
NEWS 34	Apr 21	New current-awareness alert (SDI) frequency in WPIDS/WPINDEX/WPIX
NEWS 35	Apr 28	RDISCLOSURE now available on STN
NEWS 36	May 05	Pharmacokinetic information and systematic chemical names added to PHAR
NEWS 37	May 15	MEDLINE file segment of TOXCENTER reloaded
NEWS 38	May 15	Supporter information for ENCOMPPAT and ENCOMPLIT updated
NEWS 39	May 16	CHEMREACT will be removed from STN
NEWS 40	May 19	Simultaneous left and right truncation added to WSCA
NEWS 41	May 19	RAPRA enhanced with new search field, simultaneous left and right truncation

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 NEWS EXPRESS  April 4 CURRENT WINDOWS VERSION IS V6.01a, CURRENT
               MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP),
               AND CURRENT DISCOVER FILE IS DATED 01 APRIL 2003
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=> s PSF
L2         3400 PSF

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AN 1992:165859 CAPLUS
DN 116:165859
TI Cytotoxicity and DNA cross-linking induced by peptide conjugated m-L-sarcolysin in human melanoma cells
AU Hansson, Johan; Lewensohn, Rolf; Ringborg, Ulrik
CS Dep. Gen. Oncol., Karolinska Hosp., Stockholm, S-104 01, Swed.
SO Anticancer Research (1991), 11(5), 1725-30
CODEN: ANTRD4; ISSN: 0250-7005
DT Journal
LA English

L5 ANSWER 2 OF 3 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1992:121167 BIOSIS
DN BA93:66967
TI CYTOTOXICITY AND DNA CROSS-LINKING INDUCED BY PEPTIDE CONJUGATED M-L SARCOLYSIN IN HUMAN MELANOMA CELLS.
AU HANSSON J; LEWENSOHN R; RINGBORG U
CS DEP. GENERAL ONCOLOGY, RADIUMHEMMET, KAROLINSKA HOSPITAL, S-104 01 STOCKHOLM 60, SWEDEN.
SO ANTICANCER RES, (1991) 11 (5), 1725-1730.
CODEN: ANTRD4. ISSN: 0250-7005.
FS BA; OLD
LA English

L5 ANSWER 3 OF 3 MEDLINE
AN 92117487 MEDLINE
DN 92117487 PubMed ID: 1768043
TI Cytotoxicity and DNA cross-linking induced by peptide conjugated m-L-sarcolysin in human melanoma cells.
AU Hansson J; Lewensohn R; Ringborg U
CS Department of General Oncology, Karolinska Hospital, Stockholm, Sweden.
SO ANTICANCER RESEARCH, (1991 Sep-Oct) 11 (5) 1725-30.
Journal code: 8102988. ISSN: 0250-7005.
CY Greece
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199202
ED Entered STN: 19920308
Last Updated on STN: 19970203
Entered Medline: 19920218

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L6 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2003 ACS
AN 2000:368410 CAPLUS
DN 132:347949
TI Method for producing **L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine** and derivatives thereof
IN Mehlem, Francesco; Di Vittorio, Pietro
PA Peptichemio A.-G., Switz.
SO PCT Int. Appl., 20 pp.
CODEN: PIXXD2
DT Patent
LA German
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000031119	A1	20000602	WO 1998-CH498	19981119

W: AU, CA, HU, IL, JP, US
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

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     EP 1129107        A1   20010905       EP 1998-952496   19981119
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     JP 2002530428     T2   20020917       JP 2000-583946   19981119
PRAI WO 1998-CH498     A    19981119
OS   CASREACT 132:347949; MARPAT 132:347949
RE.CNT  3     THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L6   ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2003 ACS
AN   1999:64700  CAPLUS
DN   130:144179
TI   Pharmaceutical composition containing Peptichemio for cancer
     treatment
IN   Mehlem, Francesco
PA   Peptichemio A.-G., Switz.
SO   PCT Int. Appl., 20 pp.
     CODEN: PIXXD2
DT   Patent
LA   German
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     JP 2001509487     T2   20010724       JP 2000-501767   19980707
     EP 1132395        A2   20010912       EP 2001-201272   19980707
     EP 1132395        A3   20020206
         R:  CH, DE, FR, GB, IT, LI
PRAI CH 1997-1651      A    19970707
     EP 1998-929194    A3   19980707
     WO 1998-CH300     W    19980707
RE.CNT  5     THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
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British Journal of Cancer, 1998, vol. 78 (3) pp. 328-335 ISSN: 0007-0920

The 8226/Dox$_{40}$ was selected for doxorubicin resistance and shows the classical MDR phenotype with overexpression of P-glycoprotein 170 (Dalton et al, 1986). The 8226/LR-5 was selected for Mel resistance, proposed to be associated with increased levels of glutathione (Bellamy et al, 1991; Mulcahy et al, 1994). The U-937-Vcr was selected for vincristine resistance, proposed to be tubulin associated (Botling et al, 1994). The H69AR, selected for doxorubicin resistance, expresses a multidrug-resistant (MDR) phenotype proposed to be mediated by a multidrug resistance-associated protein (MRP$_1$, Mirski et al, 1987; Cole et al, 1992). The CEM/VM-1, selected for teniposide resistance, expresses an atypical MDR, which is proposed to be topoisomerase II (topoII) associated (Danks et al, 1987, 1988). The exact mechanism of resistance for the primary resistant ACHN cell line is not known and may be multifactorial (Nygren and Larsson, 1990).

The cell lines were grown in complete culture medium described below at 37°C in humidified atmosphere containing 5% carbon dioxide. The 8226/Dox$_{40}$ was treated once a month with doxorubicin at 0.24 μg ml^{-1} and the 8226/LR-5 at each change of medium with Mel at 1.53 μg ml^{-1}. The U-937-Vcr was continuously cultured in the presence of 10 ng ml^{-1} vincristine and the H69AR was alternately fed with drug-free medium and medium containing 0.46 μg ml^{-1} doxorubicin. The CEM/VM-1 cell line was cultured in drug-free medium without any loss of resistance for a period of 6–8 months. The resistance patterns of the cell lines were routinely confirmed in control experiments.

Patient samples

A total of 49 patient tumour samples from the different diagnostic group was used to determine the activity of P2, Mel, and, for comparison, five other cytotoxic drugs were chosen to represent different mechanistic classes. However, because of a limited number of cells, all drugs could not be tested in all samples. Twenty-eight solid and 21 haematological tumours were used to determine the dose–response relationship for P2 and Mel. The diagnostic groups of origin were: acute lymphocytic leukaemia (seven), acute myelocytic leukaemia (eight), chronic lymphocytic leukaemia (four), myeloma (two), carcinoma of the bladder (one), breast cancer (four), non-small-cell lung cancer (six), ovarian carcinoma (eight), phaeochromocytoma (one), sarcoma (two), carcinoma of the thyroid (one), mesothelioma (one), unknown primary (one), gastric cancer (one), cardiac carcinoma (one), neuroblastoma (one). The overall percentage of previously untreated patients was 58%. Five samples of normal peripheral blood mononuclear cells (PBMCs) from healthy blood donors were compared with those of the five chronic lymphocytic leukaemia (CLL) samples.

The tumour samples were obtained by bone marrow/peripheral blood sampling, routine surgery or diagnostic biopsy, and this sampling was approved by the local ethics committee at the Uppsala University Hospital. Leukaemic cells and PBMCs were isolated from bone marrow or peripheral blood by 1.077 g ml^{-1} Ficoll–Paque (Kabi-Pharmacia, Uppsala, Sweden) density gradient centrifugation (Larsson et al, 1992). Tumour tissue from solid tumour samples was minced into small pieces and tumour cells were then isolated by collagenase dispersion followed by Percoll (Kabi-Pharmacia) density gradient centrifugation (Csoka et al, 1994). Cell viability was determined by the trypan blue exclusion test and the proportion of tumour cells in the preparation was judged by inspection of May–Grünwald–Giemsa-stained cytospin preparations by a cytopathologist. In some cases, cells were cryopreserved in a culture medium containing 10% dimethylsulphoxide (DMSO; Sigma Chemical Co., St Louis, MO, USA) and 50% inactivated fetal calf serum (FCS; HyClone, Cramlington, UK) by initial freezing for 24 h at –70°C, followed by storage in liquid nitrogen or in the deep freeze at –150°C. Cryopreservation in this way does not affect drug sensitivity (Nygren et al, 1992).

Table 1 Chemical composition of peptichemio oligopeptides

Peptide 1 (P1): L-Ser-LpFPhe-L-mSL.OEt
Peptide 2 (P2): L-Pro-L-mSL-LpFPhe.OEt
Peptide 3 (P3): L-pFPhe-L-mSL-Asn.OEt
Peptide 4 (P4):L-mSL-L-Arg(NO_2)-L-Nval.OEt
Peptide 5 (P5): L-pFPhe-Gly-L-mSL-L-Nval.OEt
Peptide 6 (P6): L-mSL-L-Arg-L-Lys-L-mSL-L-His.OMe

Reagents and drugs

Fluorescein diacetate (FDA; Sigma) was dissolved in DMSO and kept frozen (–20°C) as a stock solution protected from light. A complete medium consisting of culture medium RPMI-1640 (HyClone, Cramlington, UK) supplemented with 10% inactivated FCS, 2 mM glutamine, 50 μg ml^{-1} streptomycin and 60 μg ml^{-1} penicillin was used throughout for both cell lines and patient samples. Mel was obtained from the Wellcome Foundation, London, UK. The drug was received as a sterile powder, 2 mg of which were dissolved in 0.5–1 ml of 92% ethanol with 2% hydrogen chloride and further diluted in cell culture medium to the desired drug concentrations. The components of PTC and m-L-m-L-SL were obtained from Istituto Sieroterapico, Milanese, S. Belfanti, Milan, Italy. The peptides 1–5 were obtained as ethyl esters and peptide 6 as methyl ester (Table 1). An aliquot of 2 mg of each was dissolved in 0.5–1 ml of 92% ethanol with hydrochloric acid and further diluted in cell culture medium to the desired drug concentrations. Cisplatin, cytarabine, doxorubicin, etoposide and vincristine were obtained from commercial sources and were dissolved according to guidelines from the manufacturer and further diluted in phosphate-buffered saline (PBS; HyClone) or sterile water.

In the cell line panel all drugs were tested at four different drug concentrations, obtained by fivefold serial dilution from the maximum 10 μg ml^{-1}. On a molar basis the concentration of the different oligopetides are 39–43% of that of Mel and m-L-SL. To determine the dose–response relationship for Mel and P2 in patient samples, five different drug concentrations were used, obtained by a fivefold serial dilution of the drugs from 50 μg ml^{-1}. In the patient samples, the concentrations chosen for comparison with standard drugs were the empirically derived cut-off concentrations (EDCCs), defined as the concentration that produces a significant scatter of survival index (SI) values among haematological tumours. This concentration was used to optimize the conditions for evaluating cross-resistance. The concentrations 2 and 0.08 μg ml^{-1} were chosen for Mel and P2, respectively, and the EDCCs for the other drugs have been described previously (Larsson et al, 1992).

Ninety-six-well microtitre plates (Nunc, Roskilde, Denmark) were prepared with 20 μl per well of drug solution at ten times the desired concentration, with the aid of a programmable pipetting robot (Propette, Perkin Elmer, Norwalk, CT, USA). The plates

were stored frozen at –70°C for up to 2 months until further use. Under these conditions, no apparent change in drug activity was observed (Larsson et al, 1992).

The fluorometric microculture cytotoxicity assay procedure

The fluorometric microculture cytotoxicity assay (FMCA) is based on measurement of fluorescence generated from hydrolysis of FDA to fluorescein by cells with intact plasma membranes and has been described in detail previously (Larsson et al, 1992). Briefly, the cells were resuspended in complete medium, and 180 µl of cell suspension was seeded into the wells of 96-well experimental microtitre plates prepared with drugs as described. Cell densities were 5–20 × 10^3 cells per well for the cell lines, 10–20 × 10^3 cells per well for the solid tumour cells and 50–100 × 10^3 cells per well for the haematological tumour cells. Each drug and concentration was tested in triplicate. Six wells with cells but without drugs served as control and six wells with only culture medium as blank.

The plates were incubated for 72 h at 37°C in humidified conditions containing 5% carbon dioxide. At the end of the incubation period the plates were centrifuged (200 *g*, 5 min) and the medium was removed by aspiration. After one wash in PBS, 100 µl per well of FDA dissolved in PBS (10 µg ml^{-1}) was added. The plates were incubated for 45 min and the generated fluorescence (excitation 480 nm) from each well was measured at 538 nm in a 96-well scanning fluorometer (Fluoroscan II, Labsystems Oy, Helsinki, Finland). The fluorescence is proportional to the number of intact cells in the well.

To evaluate the schedule dependency of drug activity, CCRF-CEM cells and ACHN cells were used and were exposed to the drug for 2, 4 or 72 h followed by washing with PBS, addition of new culture medium and analysis at 72 h. Stability of P2 and Mel under assay conditions was investigated by a bioassay. Plates prepared with Mel and P2 were preincubated with 100 µl medium per well for different time periods, ranging from 0 to 72 h, at 37°C before cell suspension (U-937-GTB) was added. The activity of the drugs after different preincubation times was evaluated by comparing the SI values obtained after a further 72 h incubation with FMCA, as described above.

Quality control

Quality criteria for a successful analysis included a fluorescence signal in the control wells of more than five times mean blank value, a mean coefficient of variation (CV) in the control wells of less than 30% and more than 70% tumor cells in the cell preparation before incubation.

Quantification of FMCA results

Cell survival is presented as survival index (SI), defined as the fluorescence in experimental wells in per cent of that in control wells, with blank values subtracted. The IC_{50} was defined as the concentration giving a SI of 50%.

For both cell lines and primary cultures, the IC_{50}s were evaluated for each individual cell line and drug with custom-made computer software (Dhar et al, 1996). A delta value was calculated as the logarithm of the IC_{50} of the individual cell line minus the mean of all ten log IC_{50}s (Fridborg et al, 1996). The resistance factors (RFs) in each subline were defined as the IC_{50} of the resistant subline divided by the IC_{50} of its sensitive parental cell line. The pairs of parental/resistant cell lines used for RF calculations of P-glycoprotein (P-gp), MRP, topo II, glutathione (GSH) and tubulin-associated resistance were RPMI 8226S/8226Dox40, NCI-H69/H69AR, CCRF-CEM/CEM-VM-1, RPMI 8226S/8226LR-5 and U-937-GTB/U-937-Vcr respectively. Correlation coefficients were determined using Pearson's correlation coefficient. Response rate was defined as the fraction of samples having a SI below 50% at 0.5 µg ml^{-1} for all samples investigated. In vitro therapeutic index was calculated as median IC_{50} of CLL samples/median IC_{50} of normal PBMCs.

Measurement of DNA synthesis

In some experiments bromodeoxyuridine (BrdU) incorporation into cellular DNA was determined with an enzyme-linked immunosorbent assay (ELISA) kit from Boehringer Mannheim (Mannheim, Germany) essentially according to the protocol provided by the manufacturer. Briefly, cells were incubated in 96-well plates for 72 h in the presence of BrdU. The cells were then fixed and an antibody directed to BrdU was added. The formed immune complex was detected by a substrate reaction using tetramethylbenzidine and measured in a spectrophotometric microplate reader (Dynatech, Billingshurst, UK).

RESULTS

Activity patterns of PTC oligopeptides in the cell line panel resembles that of Mel

Concentration–response curves for Mel in the cell line panel are shown in Figure 1A. Delta, the deviation of log IC_{50} from the mean log IC_{50} of the cell line panel, is shown in Figure 1B. When the patterns of deltas for Mel were compared with those of m-L-SL and the components of PTC using Pearson's correlation analysis, a high correlation was obtained for several of the m-L-SL oligopeptides (Table 2). For m-L-SL, P1, P2 and P4 the correlation coefficients were > 0.90. No correlation was established with P6 as an IC_{50} was reached in only one cell line.

P2 is more potent than the other PTC oligopeptides

P2 was the most active m-L-SL oligopeptide, which showed a slightly lower mean IC_{50} (2.6 µg ml^{-1}) compared with Mel (3.9 µg ml^{-1}) and m-L-SL (4.1 µg ml^{-1}). However, on a molar basis, the IC_{50} value for P2 was 3.3 times lower than m-L-SL. P1 showed an IC_{50} of 4.1 µg ml^{-1} whereas the remaining m-L-SL oligopeptides had IC_{50}s between 5.8 and 9.1 (Table 2). P2 was the only oligopeptide producing a SI below 50% in all the tested cell lines (Table 2).

P2 appears not to be affected by GSH-associated resistance

Although, the overall activity profile resembled that of Mel, P2 appeared not to be affected by GSH-associated resistance as determined by the low resistance factor obtained using the LR5-parental IC_{50} ratio (RF 1.05, Table 3). Mel and m-L-SL, on the other hand, showed RFs of 3.1 and 3.8 respectively. P2 also appeared less sensitive to MRP-associated resistance than Mel and m-L-SL with RFs of 1.55, 4.0 and 4.17 respectively (Table 3). Neither of the drugs was affected by the remaining resistance mechanisms.

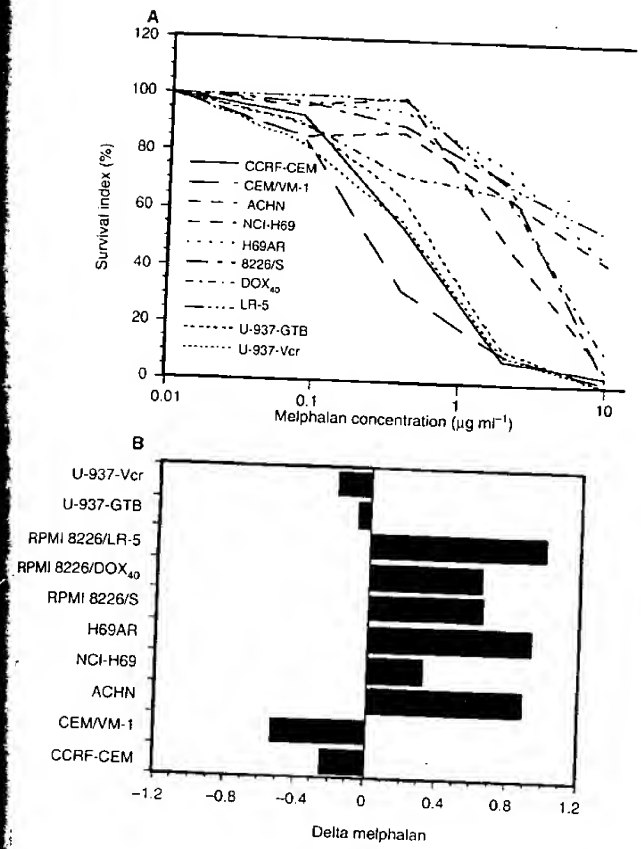

Figure 1 Effect of Mel on survival index (SI) for all investigated cell lines. Survival index: fluorescence in test wells/fluorescence in control wells with blank values subtracted (**A**). From these concentration–response curves, mean $\log_{10}$ IC_{50} was determined defined as the mean of the $\log_{10}$ values of all ten individual IC_{50}s obtained for the drug. Then, the difference between the $\log_{10}$ of each cell line and the mean $\log_{10}$ IC_{50} was calculated to yield a variable defined as delta (*x*-axis). A mean graph consisting of the drug-specific deltas across the cell line panel could then be constructed to visualize differential cytotoxicity patterns of drugs (**B**). Thus, bars projecting to the left (negative values) indicate cell lines more sensitive than the average and bars projecting to the right (positive values) indicate drugs more resistant than the average for a particular drug. See also Materials and methods

P2 is more active than Mel against primary human tumour cells from patients

The activity of P2 and Mel was then further characterized in 49 fresh human tumour samples, 21 from haematological and 28 from solid tumour patients. In these samples P2 was considerably more active than Mel, showing IC_{50} values of 0.51 and 8.6 compared with 2.3 and 23.8 μg ml^{-1} for haematological and solid tumour samples respectively (Figure 2). When compared with the cell lines, P2 was significantly more active against the primary cultures, showing an IC_{50} ratio for Mel over P2 of 11.2 compared with 1.5 for the cell lines. A tendency towards higher relative solid tumour activity for P2 was also observed, two and six solid tumour samples showing negative deltas compared with overall mean value for P2 and Mel respectively (Figure 2).

The six solid tumour samples were from patients with ovarian cancer (two), neuroblastoma, non-small-cell lung cancer, breast cancer and carcinoid tumour. At clinically achievable exposure for

Table 2 Results of comparative testing of Mel (melphalan) and the related compounds in a mechanism-based cell line panel.[a]

Rank	Drug	IC_{50}[a] mean	R[b]	IC_{50} max	IC_{50} min
1	Mel	3.9	1.0	10	0.29
2	m-L-SL	4.1	0.99	9.1	0.61
3	P1	4.1	0.97	10	1.1
4	P4	6.8	0.92	10	1.8
5	P2	2.6	0.90	5.3	0.38
6	P5	5.8	0.81	10	1.5
7	P3	7.6	0.69	10	1.6
8	P6	9.1	nd	10	1.2

[a]For the ten cell lines depicted in Figure 1 mean IC_{50}s were determined for all oligopeptides and the results are expressed as μg ml^{-1}. [b]Correlations of the cell line panel log IC_{50} values using Mel as the reference compound.

Table 3 Resistance factors for Mel (melphalan), m-L-SL (Sarcolysine) and P2

Resistance mechanism	Resistance factors (RF)[a] Mel	m-L-SL	P2
P-gp-associated MDR	0.99	0.95	0.96
Topo II-associated MDR	0.52	0.76	0.69
Tubulin-associated MDR	0.75	0.96	1.02
GSH-associated MDR	3.10	3.80	1.05
MRP-associated MDR	4.0	4.17	1.55

[a]Resistance factor (RF) = IC_{50} in resistant cell line/IC_{50} in parental cell line. Results are presented as one typical experiment out of three.

Mel (2.0 μg ml^{-1}) an in vitro response rate (percentage of samples with > 50% decrease in SI) of 67% and 0% was observed for haematological and solid tumour samples respectively. The corresponding response rates for P2 was 100% and 43% (Table 4).

P2 is more active than Mel on low-proliferating tumour cell systems

To investigate whether the increased activity of P2 could be related to the low proliferative rate of the primary cultures, the ratio of Mel vs P2 IC_{50}s in the cell lines was plotted against the rate of proliferation under assay conditions in V-shaped plates (Figure 3). An inverse relationship was observed (R = 0.70), P2 being more active against the low-proliferating cell lines. The next series of experiments aimed to determine whether this relationship was causally related to proliferation rather than being cell-type specific. ACHN, which shows a low growth rate in V-shaped plates but proliferates rapidly when seeded into flat-bottomed plates, was used for this purpose. When tested in flat-bottomed plates P2, Mel and m-L-SL showed similar IC_{50}s (not shown). In V-shaped plates, on the other hand, the corresponding IC_{50} for Mel and m-L-SL was significantly increased (four- to fivefold), whereas, by comparison P2 retained much of its activity (< two fold, not shown). Stability under assay conditions determined by a bioassay was similar for Mel and P2 (half-life of approximately 2 h, not shown) and 2-, 4- and 72-h exposure times showed similar relative concentration–response relationships for the two drugs (not shown).

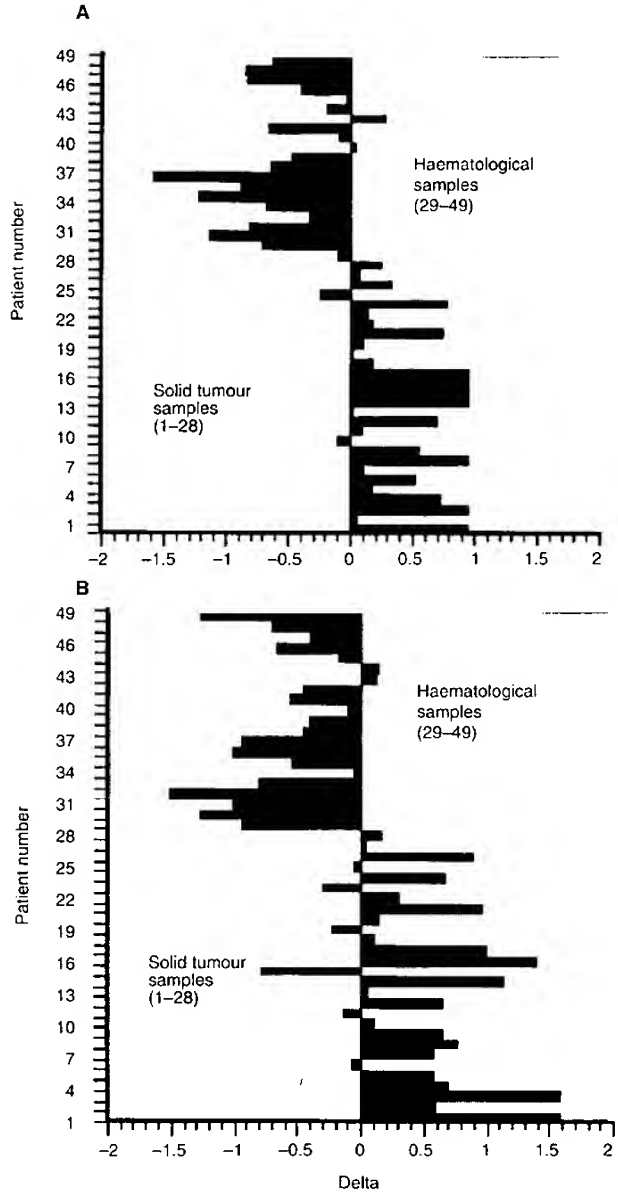

Figure 2 From concentration–response curves of Mel (**A**) and P2 (**B**) obtained from 49 primary human tumour cell samples (21 haematological and 28 solid tumour samples), mean $\log_{10}$ IC_{50} was determined defined as the mean of the $\log_{10}$ values of all 49 individual IC_{50}s obtained for the drug. Then, the difference between the $\log_{10}$ of each tumour cell sample and the mean $\log_{10}$ IC_{50} was calculated to yield a variable defined as delta (*x*-axis). A mean graph consisting of the drug-specific deltas across the cell line panel could then be constructed to visualize differential cytotoxicity patterns of drugs (**B**). Thus, bars projecting to the left (negative values) indicate tumour samples more sensitive than the average and bars projecting to the right (positive values) indicate drugs more resistant than the average for a particular drug. See also Materials and methods

P2 shows a low degree of cross-resistance to standard agents

Not only in the cell line panel (Table 2) but also in the primary cultures (Figure 4), was the correlation between P2 and Mel relatively high, indicating a similar mode of action. However, cross-resistance to standard drugs determined using the haematological samples was generally low (0.14–0.43, Table 5). The correlation with doxorubicin, etoposide and cisplatin was much lower for P2 than Mel, whereas the correlations with cytarabine and vincristine were of similar magnitude (Table 5)

Table 4 In vitro activity of Mel and P2 on primary cultures of human tumour cells from patients with haematological and solid tumours.

	IC_{50} (s.d.)		Response rate (%)[a]		
Tumour type	P2	Mel	P2	Mel	*n*
Haematological tumours	0.51 (0.52)	2.3 (2.6)	100	67	21
Solid tumours	8.6 (13.4)	23.8 (18.8)	43	0	28
Total	5.2 (10.9)	14.6 (17.8)	67	28	49

[a]Response rate was defined as the number of samples with >50% decrease in SI/total number of samples × 100 at 2 μg ml^{-1} for each drug.

P2 shows a similar in vitro therapeutic index to Mel in low-proliferating cell systems

P2 also showed lower IC_{50}s than Mel in PBMCs with median values of 0.27 and 4.0 respectively (n = 5). However, when compared with median IC_{50} values of malignant CLL samples, 0.07 and 1.4 μg ml^{-1} respectively (n = 5) the in vitro therapeutic index (IC_{50} PBMCs/IC_{50} CLL) was 3.9 for P2 and 2.8 for Mel (Table 6).

DISCUSSION

Mel and m-L-SL are closely related aromatic nitrogen mustard derivatives. The two molecules differ only in the position of the di-(2-chloroethyl) amino-group, which is in the para position in Mel and in the meta position in m-L-SL. By conjugation of additional amino acids to the carboxyl and amino groups of m-L-SL, a complex consisting of six different peptides has been developed. This mixture of peptides, PTC, has shown clinical activity in several human malignancies (Hug et al, 1980; Paccagnella et al, 1986; Zaniboni et al, 1988). In previous investigations with the human melanoma cell line RPMI 8322, PTC as well as some of its individual peptides were more effective than Mel and m-L-SL (Lewensohn et al, 1991*a*; Hansson et al, 1991). Myeloma cells isolated from bone marrow of patients with primary myelomas were more sensitive to PTC than to Mel (Paccagnella et al, 1985). In the human melanoma cell line RPMI 8322, used in the above-mentioned investigation, we found that one of the peptides in PTC, L-prolyl-m-L-sarcolysyl-L-p-fluorophenylalanine (P2), showed a higher toxicity than free m-L-SL as measured by the clonogenic assay (Hansson et al, 1991).

In the present study we show that P2 was the most active component of PTC when tested in a panel of human tumour cell lines. Moreover, P2 was also more active than Mel and m-L-SL against several of the cell lines. Correlation analysis of cell line panel activity patterns demonstrated a close relationship between P2 and Mel, suggesting a similar mode of action. However, unlike Mel and m-L-SL, P2 appeared not to be affected by GSH- and MRP-associated resistance to any greater extent. We have previously found, using a clonogenic assay, that buthionine sulphoximine, which depletes GSH, sensitizes a melanoma cell line to Mel but to a lesser extent to P2 (Hansson et al, 1991). The previous results with BSO as well as the present, showing low RFs for GSH-mediated resistance, may indicate less dependence on

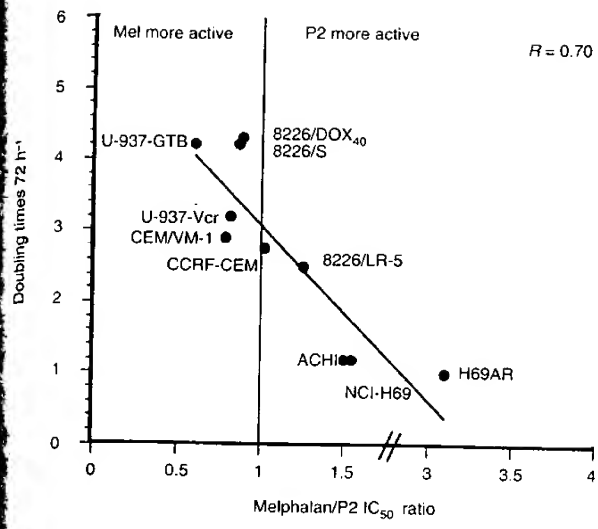

Figure 3 Relationship between Mel/P2 IC_{50} ratios and number of doubling times 72 h^{-1} determined by haemocytometer counts in the cell line panel ($n = 10$). R = Pearson's correlation coefficient

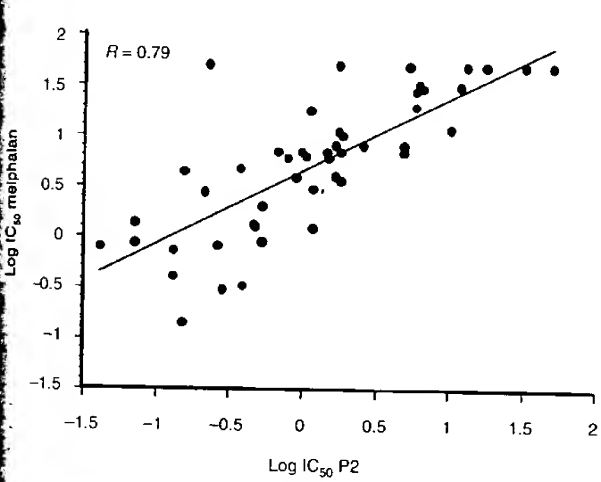

Figure 4 Correlation between log IC_{50} values for Mel and P2 in 49 primary human tumour samples. R = Pearson's correlation coefficient

cellular GSH levels for P2 than Mel sensitivity. The explanation for the lack of GSH-mediated resistance in reponse to P2 does not appear to involve intracellular liberation of m-L-SL as this compound shows RFs of similar magnitude to Mel for GSH-associated resistance. Altered substrate recognition of m-L-SL oligopeptides by cellular GSH-dependent enzymes is one potential explanation for the phenomenon. In vitro sensitivity to Mel has previously been noted to result in only a limited increase in toxicity exerted by PTC as compared with Mel in freshly obtained bone marrow myeloma cells from untreated patients.

Interestingly, this finding was contrasted by the relatively pronounced sensitivity to PTC in cell populations with in vitro resistance to Mel (Lewensohn et al, 1991*b*). When comparing Mel, m-L-SL, PTC and P2 on freshly obtained myeloma cells, P2 displayed the highest activity (data not shown).

Table 5 Correlation of Mel and P2 with standard drugs in haematological tumour cell samples from patients at EDCC

Compound	Mel (R)[a]	P	P2(R)	P	n
Doxorubicin	0.63	<0.01	0.32	NS	20
Vincristine	0.36	NS[b]	0.43	NS	20
Etoposide	0.45	NS	0.14	NS	19
Cytarabine	0.39	NS	0.37	NS	20
Cisplatin	0.58	<0.05	0.24	NS	14

[a]Pearson's correlation coefficient. [b]NS, not significant ($P > 0.05$).

Table 6 Comparison of median IC_{50}s in CLL and normal PBMC samples for P2 and Mel

Cell type	IC_{50} P2	IC_{50} Mel
PBMC ($n = 5$)	0.27	4.0
CLL ($n = 5$)	0.07	1.4
Ratio PBMC/CLL	3.9	2.8

PBMC, peripheral blood mononuclear cell; CLL, chronic lymphocytic leukaemia.

In contrast to proliferating cell lines, human tumour biopsy cells were as a group significantly more sensitive to the cytotoxic activity of P2 than Mel. The reason for this may be related to the low proliferative activity of the primary cultures in the present assay system (Weisenthal et al, 1991) as low-proliferating cell lines also showed higher relative P2 sensitivity. Furthermore, direct manipulation of the proliferative rate of the ACHN cell line produced the corresponding alterations of Mel vs P2 sensitivity. From a clinical point of view, the demonstrated ability of P2 to retain activity against non-cycling cells may be a distinct advantage as the low growth fraction of many solid tumours is a limiting factor for therapeutic responses of most currently used antineoplastic agents. The indications of a wider spectrum of anti-tumour activity and a favourable therapeutic index in vitro as well as the low cross-resistance with standard agents clearly adds to the potential of P2 being a clinically useful anti-tumour agent. What then is the mechanism for increased toxicity of P2 against primary cultures and other non-proliferating cell systems? Although, the drug appears to act mechanistically similar to Mel both in the cell lines and the primary cultures, one may speculate on, at least, two possible explanations. On one hand the effect of a bifunctional alkylating agent is related to the frequency of DNA damage such as DNA cross-links (Lewensohn et al, 1991*a*). The frequency of DNA cross-links may, however, be regulated by DNA repair mechanisms, which at least in some cell lines is correlated with drug sensitivity (Batist et al, 1989). It would then seem possible that a bifunctional alylating agent in the form of an oligopeptide would not be recognized and excised from the DNA by the same repair mechanism as Mel. On the other hand another possible explanation is that of a more effective cellular uptake of the bifunctional alkylator when in the form of an oligopeptide as compared with Mel only. In this context, it is interesting to note that another tripeptide of m-L-SL, 3-(p-fluorophenyl)-L-alanyl-3-[m-bis(2-chloroethyl) aminophenyl]-L-alanyl-L-methionine ethyl ester], PTT.119, has shown increased anti-tumour activity (Yagi et al, 1984, 1988) and the delivery of this peptide into tumour cells was found to be significantly greater than Mel. It was subsequently

found that this peptide used multiple transport pathways in L1210 cells (Yagi et al, 1988). Both the above alternatives are currently being explored.

In whole blood P2 is rapidly degraded to m-L-SL, a fact that may limit the activity of the drug in vivo (Ehrsson et al, 1993). This finding indicates that peptidase activity probably degrades the P2 compound intracellularly. Degradation of di-, tri- and tetrapeptides has previously been observed in erythrocytes and leucocytes that have high peptidase activity (Stern et al, 1951). More attempts will be made to characterize exactly the intracellular degradation of P2 and test its efficacy in comparison with m-L-SL in vivo.

In the present study, we used a human cell line panel in combination with a panel of primary tumour cultures from patients for in vitro evaluation of differential drug responses of PTC oligopeptides. In a previous study (Dhar et al, 1996) we showed that the present cell line panel is capable of detecting mechanisms of action of standard drugs in addition to its ability to evaluate sensitivity to drugs to defined types of mechanisms of resistance. Complementary to this, non-clonogenic assays used on fresh primary tumour cultures from patients have been shown to mimic the known clinical activity pattern of standard drugs. We have also previously shown that non-clonogenic cytotoxicity assays such as the FMCA can detect tumour type specific activity retrospectively for a series of standard drugs (Nygren et al, 1994) and prospectively for early phase I–II drugs such as vinorelbine, idarubicin, CdA, gemcitabine, taxol and topotecan (Larsson et al, 1994; Larsson and Nygren, 1994; Csoka et al, 1995; Nygren et al, 1995; Fridborg et al, 1996; Jonsson et al, 1997). Thus, experience gained so far suggests that these model systems may be valid tools for initial predictions of the activity and potential utility of novel anticancer drugs.

In summary, we have demonstrated high anti-tumour activity of a m-L-SL oligopeptide against cell lines and primary cultures of tumour cells from patients. The drug appears to show retained activity against non-proliferating cell systems, shows a positive therapeutic index and demonstrates low levels of cross-resistance with standard drugs. Formal testing of these in vitro predictions will require in vivo testing in relevant tumour models and these studies are currently under way.

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CYDEX AND PEPTICHEMIO ANNOUNCE
THE SIGNING OF A CAPTISOL® LICENSING AGREEMENT

(OVERLAND PARK, KS, June 14, 1999) - CyDex, Inc. and Peptichemio, AG today announced the signing of a licensing agreement granting Peptichemio worldwide rights to CyDex's CAPTISOL® sulfobutylether beta-cyclodextrin (SBE-b-CD) drug delivery technology for Peptichemio's unique anticancer compound. Under the license agreement, Peptichemio will conduct initial clinical trials utilizing CyDex's CAPTISOL® 's drug delivery technology to deliver their drug. Economic terms of the agreement were not announced.

Peter Higuchi, President and CEO of CyDex said, " We are pleased to be working with Peptichemio on this exciting opportunity. It affords us the opportunity to partner with another small company and to create additional value for the both of us."

"We evaluated several delivery systems and CyDex's CAPTISOL® proved superior. The animal results we obtained on our compound are very encouraging, and we are very pleased that CyDex is willing to partner with us to further develop our technology," said Dr. Victor E. Hofmann, CEO of Peptichemio.

CyDex, Inc., a privately owned company located in Overland Park, Kansas, was established to license, develop and commercialize a series of anionically charged sulfobutylether beta-cyclodextrins originally synthesized and patented by scientists from the University of Kansas Higuchi Biosciences Center for Drug Delivery Research for use in drug development and formulation. CAPTISOL, a modified anionic beta-cyclodextrin derivative, is a donut shaped molecule with a hydrophilic outer surface and a lipophilic cavity. CAPTISOL can complex some poorly water soluble drug compounds in its lipophilic cavity, producing a CAPTISOL/drug complex that is more water soluble than the drug alone. The CAPTISOL/drug complex can then be formulated and administered to patients where the complex disassociates, allowing the drug to produce its desired pharmacological activity.

Peptichemio, AG is a closely held private Swiss company headquartered in Bern Switzerland. Peptichemio is developing a unique alkylating agent linked to a specific protein carrier that targets the alkylating agent's activity and reduces systemic side effects. Peptichemio has generated preclinical animal data showing encouraging anticancer activity.

CAPTISOL is a registered trademark of CyDex, Inc.

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(54) Title: CYCLODEXTRIN-PEPTIDE COMPOSITIONS

(57) Abstract

This invention provides improved compositions containing cyclodextrin complexes of peptides, particularly synthetic peptides and peptides of ≤ 40 amino acids. Such peptides are particularly useful for administration as receptor agonists, receptor antagonists, and as vaccines. The compositions of the invention provide improved means for delivery of such peptides.

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CYCLODEXTRIN-PEPTIDE COMPOSITIONS

Field of the Invention:

This invention relates to a method of presenting pharmaceutically active peptides, particularly receptor blockers and immunogenic peptides, in cyclodextrin compositions.

Background of the Invention:

Cyclodextrins are cyclic molecules containing six or more α-D-glucopyranose units linked together at the 1,4 positions. The 2-hydoxypropyl-ß-cyclodextrin (HPCD) has been used for stabilization and solubilization of various compounds, including proteins and steroids. Brewster, et al. described use of cyclodextrins in solubilizing proteins to prevent aggregation , precipitation, and loss of biopotency. ("Application of 2-hydroxypropyl beta cyclodextrin to Proteins", Minutes Int. Symp. Cyclodextrins, 5th, 1990, pp 440-444) The proteins studied therein were interleukin-3, and insulin, two large regulatory proteins. There is no suggestion that the 2-hydroxypropyl beta cyclodextrin would be useful in formulating peptides for use as receptor blockers or immunogens. The Brewster article suggests that the improved potency of the proteins is due to the avoidance of hydrolysis, deamidation, racemization, oxidation and disulfide bond exchange, and changes in dimensional protein structure related to folding of the protein. There is no suggestion that the cyclodextrins can be useful for formulations containing synthetic peptides, nor is there any suggestion that the preparations disclosed therein can be administered by application to the mucosa.

Josef Pitha, in U.S. patent 4,727,064, which is incorporated herein by reference, suggests the use of cyclodextrin in solubilizing medicinals including steroids and vitamins, but does not disclose the solubilization of peptides in cyclodextrin.

Szejtli, et al., in U.S. patent 4,380,626, teach the use

of cyclodextrins in preparations of plant growth regulators including 2-chloroethylphosphonic acid. No use of cyclodextrins for preparation of peptides is taught or suggested therein.

Gideon Goldstein, in U.S. Patent 5,140,010, which is incorporated herein by reference, teaches the stabilization of aqueous formulations of synthetic peptides corresponding to position 32-36 of thymopoietin and known as thymopentin or TP-5 (Arg-Lys-Asp-Val-Tyr) in glycine. Goldstein does not disclose or suggest use of cyclodextrin for stabilization of peptides. TP-5 is effective in blocking the stimulation of smooth muscle contraction caused by the neurotoxin (+)-anatoxin-a (ANTX). ANTX is a bicyclic amine exotoxin produced by the blue-green algae, Anabaena flos-aquae, and has been found to cause death to livestock and waterfowl. The toxin acts by depolarizing blockade of neuromuscular transmission. Such depolarization results in respiratory paralysis. The action of ANTX has been ascribed to its potent nicotinic cholinergic agonist activities in skeletal muscle and mammalian skeletal muscle and the central nervous system. ANTX can also cause cardiovascular aberrations by activation of nicotinic receptors in the adrenal medulla and sympathetic ganglia. The antagonist effect of TP-5 has been attributed to its ability to block nicotinic receptors in a noncompetitive manner.

Summary of the Invention:

This invention provides improved compositions containing cyclodextrin complexes of peptides, particularly synthetic peptides and peptides of $\leq$ 40 amino acids. Such peptides are particularly useful for administration as receptor agonists, receptor antagonists, and as vaccines. The compositions of the invention provide improved means for delivery of such peptides.

Many peptides, especially peptides of about three to 20 amino acids, are unstable in low concentrations and tend to loose biological activity. While Brewster describes the value of preparing formulations of cyclodextrin and regulatory proteins to avoid conformational changes, there is no suggestion therein that cyclodextrin would be useful for increasing

stability of small peptides such as thymopentin.

The instant invention improves methods of administration of peptides to the mucosa of mammals in need of treatment with effective peptides.

Detailed Description of the Invention:

The invention provides a means of formulating peptides to avoid loss of efficacy and to facilitate delivery of the active peptides to the reactive site. The method has been exemplified using the synthetic peptides corresponding position 32-36 of thymopoietin and known as TP-5 (Arg-Lys-Asp-Val-Tyr). While the hydroxypropyl cyclodextrin has been exemplified, other cyclodextrins, including mixed cyclodextrins, may be used in the method of the invention.

One problem in use of peptides is their instability in aqueous solution, especially very dilute compositions. Furthermore, many of the solvents used to provide stable, soluble compositions for treatment of other mammals can not be used in man. At present, there is no known compatible solvent for TP-5 in which the peptide is stable and easily administered. This is a significant problem because the instability will hinder acceptance for prophylactic and/or therapeutic applications.

Materials and Methods:

The 2-hydroxypropyl-ß-cyclodextrin used in the examples was purchased from Pharmatec in Alachua, Florida. TP-5 (10^{-2} M) was synthesized as describe in Chiang, et al, Life Sci 49: (1991) PL13-19 and was made up in various percentages of HPCD dissolved in sterile water. Mixtures were stirred for about one hour. The solutions were then maintained at room temperature. Control solutions of TP-5 dissolved in sterile water without HPCD were also prepared in the same manner. The two sets of solutions were stored at ambient room temperatures (25°C) for 14 months. Aliquots were removed monthly for stability testing. The stability study was performed by assaying the ability of the TP-5 solutions to counteract the stimulation of contraction of guinea pig ileum by ANTX. Guinea pig ileum contraction stimulated by ANTX was performed as

reported in Chiang, et al. (supra). The final concentration of TP-5 for use was obtained by diluting with Krebs-Ringer buffer.

EXAMPLE I

Aqueous solution of 2-hydroxy-β-cyclodextrin (HPCD) were prepared at concentration of 2.5%, 5.0%, 10%, 15%, 20%, 25% and 30% (w/v). TP-5 was added in sufficient amounts to provide a final molarity of 10^{-2} molar solution of TP-5. Further dilution to provide final dosage was made using Krebs-Ringer buffer. The solutions were then stored at ambient temperature for 14 months, after which activity of the cyclodextrin solutions was compared to freshly made solutions. As a control a 10^{-2} solution without cyclodextrin was prepared. After storage at ambient temperature (25°C) for four weeks the control solution showed no activity.

EXAMPLE II

Evaluation of Anatoxin-A Response alone and in conjunction with TP-5 was carried out in accord with standard procedures as disclosed in U.S. Patent 4,973,734 issued November 27, 1990, which is incorporated herein by reference.

Results:

IC_{50} values of the inhibition by TP-5 of guinea-pig ileum contraction stimulation by ANTX at 3 x 10^{-5} N was compared using freshly made 10_{-2} molar solutions of TP-5 and similar concentrations of TP-5 in 5%, 15% and 20% solutions of HPCD which had been stored for 14 months at ambient temperature to determine relative activity. The results are shown as mean ± s.e. of four separate experiments as indicated in Table I

TABLE I

HPCD (%)	IC_{50} (x 10^{-5} M)
0 (freshly made)	3.9 ± 1.9
5%	4.1 ± 3.1
15%	4.9 ± 4.4
20%	3.3 ± 3.0

EXAMPLE III

A composition containing 0.5 mg TP-5 is administered intraperitoneally to rabbits to provide protection against ANTX. Formulations may be administered for up to 4 days. Dosage range for thymopentin may vary from 1 μg/kg/day to 1 g/kg/day. It is, of course, understood that smaller animals will require higher dosage per kilogram than larger mammals.

Formulations of active agents in HPCD for administration may be prepared using any pharmaceutically appropriate solvent, including water, isotonic saline, glucose, or saline. The formulations may be administered orally in the form of liquid bolus, or may be administered as lyophilized powders or tablets. When provided as lyophilized powders, many of the compositions may be administered nasally for inhalation. Compositions of the invention may be administered parenterally by, for example intramuscular, subcutaneous or intraperitoneal routes. Solutions of the cyclodextrin inclusion complexes can be administered to the mucosa by any means appropriate such as by nasal spray, buccal tablet or sublingually as drops. The site of administration will be governed, in many instances, by the site of effective response. For example, it is often advantageous to administer immunogenic peptides to the mucosa.

Many other peptides could be formulated in a similar manner. Such peptides include splenopentin (SP-5) having the structure Arg-Lys-Glu-Val-Tyr. This peptide is effective for inducing T-cell differentiation and for modulation of neuromuscular transmission. (Proc. Natl. Acad. Sci. USA 81: 2847-2847 (1984)) Others include a nine amino acid sequence known as delta sleep inducing peptide (DSIP) of the structure Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu (Neurosci. Biobehav. Rev. : 83-93 (1984)), vasoactive intestinal peptide (VIP) or biotinyl-VIP from human, porcin, chick, rat or other sources, having the sequence His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2 for prevention for cell killing by human immunodeficiency virus (Nature 335: 639-642 (1984)) and for pharmacological treatment of tissues involving neuromuscular transmission (Arch. int.

Pharmacodyn 305, 14-24 (1990)). The peptide HG165-178 representing the sequence 165-178 of gp120 is represented by the sequence Asn-Ile-Ser-Thr-Ser-Ile-Arg-Gly-Lys-Val-Gln-Lys-Gln-Lys-Glu-Tyr, which is analogous to sequences in snake neurotoxins and rabies virus glycoprotein is conjugated to a keyhole limpet hemocyanin (KLH) and can be, thereafter, encapsulated in cyclodextrin to prevent the binding of viruses, toxins, viral coatings and gp120 to cells. (See FEBS Letters 311: 115-118 (1992)).

The methods of the invention should be particularly considered to stabilize peptides containing asparytyl, asparaginyl and glycine residues.

CLAIMS

1. A pharmaceutically effective composition comprising an effective amount of a receptor agonist, antagonist or immunogenic peptide of 3 to 40 amino acids in a cyclodextrin inclusion complex in a pharmaceutically acceptable diluent.

2. A composition of claim 1 wherein the immunogenic peptide is TP-5.

3. A composition of claim 1 wherein cyclodextrin is present at a concentration of .5% to 30%.

4 A composition of claim 3 wherein a cyclodextrin is 2-hydroxypropyl-ß-cyclodextrin.

5. A composition of claim 1 wherein the active peptide is an immunogen.

6. A composition of claim 1 wherein the peptide is splenopentin.

7. A composition of claim 1 wherein the peptide is delta sleep-inducing peptide.

8. A composition of claim 1 wherein the peptide is vasoactive intestinal peptide.

9. A composition of claim 1 wherein the peptide is HG 165-178.

10. A method of administering an immunogen to an animal by administering an immunogenic effective amount of a pharmaceutical composition of claim 5.

11. A method of claim 10 wherein the pharmaceutical composition is administered directly to the mucosa.

12. A method of claim 11 wherein the pharmaceutical composition is administered sublingually.

13. A method of claim 11 wherein the pharmaceutical composition is administered to the nasal mucosa by inhalation.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01847

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 39/12; C07K 3/00, 13/00, 15/00
US CL : 424/89, 85.1, 88,; 530/395, 350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/89, 85.1, 88,; 530/395, 350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, search terms: cyclodextrin, pharmaceutical, thymopentin, splenopentin, vasoactive intestinal peptide, neuronal peptide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,923,964 (GOLDSTEIN ET AL) 08 May 1990, cols. 7-10.	1-13
Y	US, A, 5,140,010 (GOLDSTEIN ET AL) 18 August 1992, see entire patent.	1-13
Y	US, A, 5,024,998 (BODOR) 18 June 1991, cols. 1-11.	1-13
Y	US, A, 4,956,274 (KHANNA ET AL) 11 September 1990, cols. 9-12.	1-13
Y	Nature, Volume 335, issued 13 October 1988, D. E. Brenneman, et al, "Neuronal Cell Killing by the Envelope Protein of HIV and its Prevention by Vasoactive Intestinal Peptide", pages 639-642, see entire article.	1-13

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proceedings National Academy Sciences, USA, Volume 81, issued May 1984, T. Audhya et al, "Contrasting Biological Activities of Thymopentin and Splenin, Two Closely related Polypeptide Products of Thymus and Spleen", pages 2847-2849, see entire article.	1-13
Y	FEBS Letters, Volume 311, No. 2, issued October 1992, L. Bracci, et al, "Binding of HIV-1 gp120 to the Nicotinic Receptor", pages 115-118, see entire article.	1-13